Photosynthesis Research **43:** 49–56, 1995.

Regular paper

Electrochromic absorbance changes in the chlorophyll-*c*-containing alga *Pleurochloris meiringensis* (Xanthophyceae)

Claudia Büchel[1] & Gyözö Garab[2]
[1]*Institute for General Botany, University of Mainz, 55099 Mainz, Germany;* [2]*Institute of Plant Biology, Biological Research Center, 6701 Szeged, Hungary*

Received 7 June 1994; accepted in revised form 23 December 1994

Key words: Xanthophyceae, Chl-*c*-containing algae, carotenoids, electrochromic absorbance changes

Abstract

Flash-induced absorbance changes were measured in the Chl-*c*-containing alga *Pleurochloris meiringensis* (Xanthophyceae) between 430 and 570 nm. In addition to the bands originating from redox changes of cytochromes, three major positive and two negative transient bands were observed both 0.7 and 20 ms after the exciting flash. These transient bands peaking at 520, 480 and 451 nm and 497 and 465 nm, respectively, could be assigned to an almost homogeneous shift of the absorbance bands with maxima at 506, 473 and 444 nm, respectively. The shape of the absorbance transients elicited from PS I or PS II was identical, and the two photosystems contributed nearly equally to the absorbance changes. Furthermore, the decay transients were sensitive to the preillumination of the cells. These data strongly suggest that the absorbance transients originate from an electrochromic response of carotenoid molecules. The pigment species responsible for the 506 nm absorption band, probably heteroxanthin or diatoxanthin, transferred excitation energy to both photosystems as shown by the aid of 77 K fluorescence excitation spectra.

Abbreviation: LHC – light-harvesting complex

Introduction

Upon illumination of photosynthetic membranes positive and negative charges are separated in the reaction centre complexes. The primary charge separation is followed by a vectorial transport of charges which is accompanied by uptake and release of protons on opposite sides of the membrane vesicle. As a result, a transmembrane electrochemical potential builds up, which consists of a proton concentration gradient, ΔpH, and an electrical potential difference, $\Delta\Psi$. By a reverse flow of protons the electrochemically stored energy is consumed in ATP synthesis (Mitchell 1974).

The rise and decay of the transmembrane electrical potential difference can be followed by observing the electrochromic absorbance change of the pigments embedded in the membrane. The pigments exposed to the electric field undergo an almost homogeneous absorption band shift, the so-called electrochromic or field-indicating shift which is proportional to the field strength (for review see Junge 1977; Witt 1979). Electrochromic absorbance changes induced by single turnover flashes provide useful information on the concentration of active reaction centres (e.g. Junge and Jackson 1982), on the secondary charge transport, e.g. in the Cyt b_6/f complex (Cramer et al. 1987), and on various factors affecting the permeability of membranes e.g. activation of ATPase (Girault and Galmiche 1976; Morita et al. 1983; Schreiber and Rienits 1982).

The detector molecules with and without permanent dipole moment respond linearly or quadratically, respectively, to a homogeneous transmembrane electric field. Local or pre-existing fields can significantly alter the sensitivity of some pigments. For example, polar chlorophyll molecules close to

carotenoids induce dipole moments in the latter which change the quadratic response to a pseudo linear field-dependence. The sensitivity of the absorbance bands of pigments also depends on the orientation of their transition dipoles with respect to the electric field vector (Paillotin and Breton 1977). Hence, different pigments do not respond equally to the electric field and in fact usually only a relatively small fraction of molecules, the 'field-indicating pigments', exhibit large, well discernible electrochromic response (Joliot and Joliot 1989). Moreover, in some membranes, e.g. in heterocysts (Houchins and Hind 1983) and unicellular cyanobacteria no flash-induced electrochromic absorbance change has been identified between 450 and 540 nm where the electrochromic bands can most easily be observed in green algae and higher plants.

In higher plants and green algae, the electrochromic shift exhibits major negative and positive maxima at 478 nm and 515 nm, respectively (Duysens 1954; Witt 1955). Schmidt et al. (1971) showed that Chl-*b* is responsible for the major negative band at 478 nm, while the transient absorbance band around 520 nm could be correlated with the interaction of Chl-*b* and lutein in the complexes (Sewe and Reich 1977).

Pleurochloris meiringensis, an alga belonging to the Xanthophyceae, contains neither Chl-*b* nor lutein. Instead of Chl-*b* a small amount of Chl-*c* is present as accessory pigment in the light-harvesting complexes. The main carotenoid diadinoxanthin is accompanied by heteroxanthin, vaucheriaxanthin-ester and diatoxanthin (Büchel and Wilhelm 1993). Thus, the electrochromic absorbance changes whose existence has not been shown in this organism are expected to be different from those in green algae and higher plants. By using light flashes of 0.3–3 s duration, light induced absorbance transients between 400 and 570 nm have already been recorded in representatives of different algal groups which do not contain Chl-*b* and lutein, including some xanthophyceae (Fork and Amesz 1967; Fork 1969; Fork and Brown 1974). The absorbance changes were suggested to originate from a shift in carotenoid absorption, a mechanism similar to the electrochromic absorbance transients in green plants.

In this work, by using single turnover flashes, we have identified the main absorbance transients between 450 and 540 nm in *P. meiringensis*. We show that the absorbance changes can be fitted with the assumption of a homogeneous shift of the absorbance bands with maxima at 506 nm, 473 nm and 444 nm, respectively. The nearly equal contribution of the two photosystems to the absorbance changes, and the sensitivity of the decay kinetics to preillumination strongly suggest that the transients originate mainly from an electrochromic shift of carotenoid molecules. The band at 506 nm could also be identified in the excitation spectrum of low temperature fluorescence emission, and the carotenoid species could be located in both LHC I and LHC II.

Materials and methods

Pleurochloris meiringensis Vischer (Culture collection Göttingen, n° 860-3) was grown as batch culture in a nutrient medium according to Böger (1969) in white light of 15 μE m^{-2} s^{-1} intensity. Cells were harvested in the logarithmic growth phase and concentrated by sedimentation. Chlorophyll content was measured after homogenisation of the cells in acetone (90%) according to Jeffrey and Humphrey (1975).

For absorption transient measurements the cells were used in nutrient solution supplemented with 0.02 M HCO_3^-; 5% Ficoll was added to avoid sedimentation of the cells during measurement. The Chl-*a* concentration of the samples was adjusted to 70 μg/ml. Unless stated otherwise, the cells were dark-adapted prior to measurements for at least 20 min. All measurements were performed at room temperature.

Absorption transients were induced by saturating flashes of red light (3 μs duration at half peak emission) in a set-up described earlier (Barabás et al. 1985). Data were collected in a digital storage oscilloscope (TEK 2224, Tektronix) and processed in a personal computer. The time resolution of the instrument was adjusted to 100 μs. Flashes were given at a frequency of 1 s^{-1} and 30 kinetic traces were averaged.

For spectral analysis, kinetic measurements were carried out at different wavelengths between 430 nm and 570 nm and analysed 700 μs after the exciting flashes. At 700 μs, between 450 and 540 nm the contributions of the absorbance changes due to redox reactions of Cyt b_6/f (Wasserman 1980) and P700 (Hiyama and Ke 1971) were small compared to the measured amplitudes. In this region the transient spectra were deconvoluted by mathematical fitting with the assumption that the transients are predominantly of electrochromic nature and thus can be fitted with a linear combination of first derivatives of gaussian absorbance bands which undergo bathochromic shift:

$$(dA/d\nu) = -(k \cdot (\nu - \nu_0)/\sigma^3 \cdot \sqrt{2\pi}) \cdot e^{\{-(\nu-\nu_0)^2/2\sigma^2\}}$$

with A = absorbance, ν = frequency at the peak position of the gaussian, and σ = halfbandwidth.

The fit of each band was performed by iteration for the halfbandwidth and peak position of the gaussian, and for the amplitude of the derivative band. The initial values of ν_0 for the iteration were obtained from the zero-crossings of the transient spectrum. For the fitting of the first band the data points between 500 and 535 nm were used. After subtracting this fit from the measured spectrum the second transient was fitted for the interval between 470 and 500 nm. This procedure was repeated for the third derivative band which was fitted for data points between 445 and 470 nm.

Fluorescence excitation spectra of whole cells at 77 K were recorded as described earlier (Büchel and Wilhelm 1993). Excitation and emission band passes were set to 1.5 and 5 nm, respectively. Spectra were recorded with a resolution of 1 data point per nm.

Results

Figure 1 shows the kinetics of flash-induced absorption changes of dark adapted and preilluminated *P. meiringensis* cells at 515 nm (A) and 553 nm (B). At 515 nm the absorbance change of dark-adapted cells was characterised by a fast (< 0.1 ms) rise followed by a slow rise peaking at around 5 ms, and a slow decay with a half decay time between 100 and 200 ms. At 553 nm, the fast absorbance decrease also relaxed relatively slowly. Upon preillumination of the cells the signals relaxed faster after the exciting flash at both 515 and 553 nm (lower traces in Fig. 1A and B). The decay of the electrochromic absorbance change at 515 nm in green algae has been shown to accelerate upon preillumination (Joliot and Delosme 1974). The rereduction of Cyt f^+, although faster in green algae than in *P. meiringensis* after dark adaptation, is also accelerated upon light-induced reduction of the plastoquinone pool (Bouges-Bocquet 1977). The similarity of the traces to data published for green algae suggested that the absorbance change at 553 nm originated from oxido-reduction of cytochromes, whereas the absorbance change at 515 nm was most probably due to an electrochromic absorbance change of pigment molecules. In *P. meiringensis*, the initial amplitude of the 515 nm signal was reduced by a factor of 10 in comparison to green algae (Joliot and Delosme 1974). On the other hand, the initial amplitude of the cytochrome signal corrected for the chlorophyll concentration was comparable to that in higher plant chloroplasts (Hope

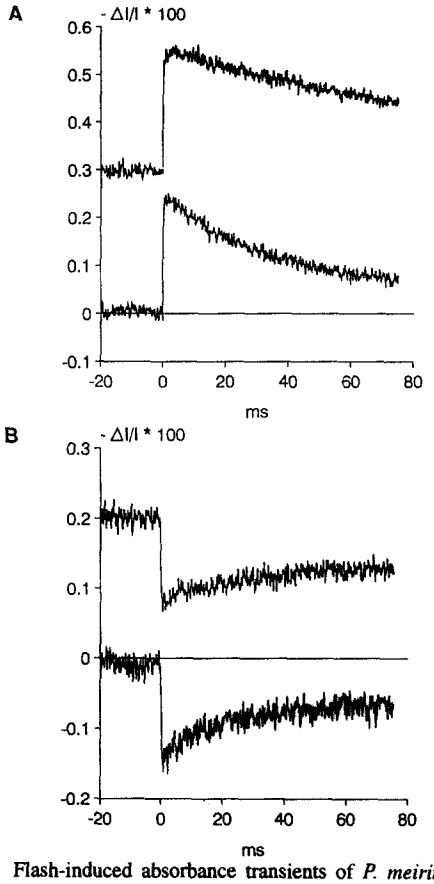

Fig. 1. Flash-induced absorbance transients of *P. meiringensis* cells at 515 nm (A) and at 553 nm (B), respectively. Upper traces were recorded after dark adaptation, the lower traces after 5 min preillumination of the cells with white light of 80 μE m^{-2} s^{-1}. In A and B the baseline of the upper traces were shifted by −0.003 and −0.002, respectively.

et al. 1992), even though under our culture conditions *P. meiringensis* contained three times less Cyt *f* on a Chl-basis than *Chlorella* or higher plants (Büchel et al. 1988; Chow et al. 1990; Wild et al. 1986). The large amplitude of the 553 nm absorbance change could be explained by the fact that in *P. meiringensis* Cyt c_{553}, which has a comparable extinction coefficient to Cyt *f* (Sandmann and Böger 1980), serves as electron donor for PS I instead of plastocyanin (Büchel and Garab 1994).

Transient spectra determined at different time intervals after the exciting flash are shown in Fig. 2. The negative peak at 553 nm, with a shoulder at 559–560 nm, could indeed be assigned to the oxidation of Cyt *f* and Cyt c_{553}. The major positive band in the transient spectra of *P. meiringensis* was located at 520 nm, close

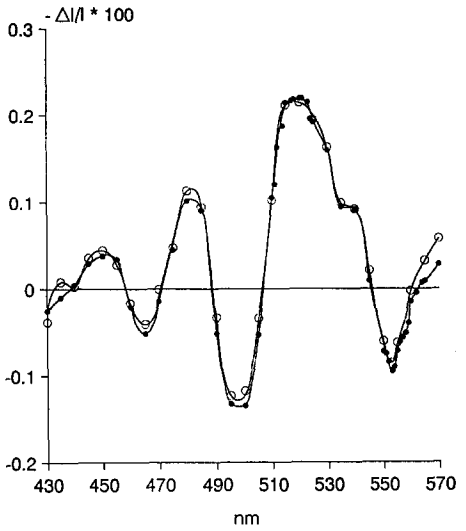

Fig. 2. Transient spectra of fast absorbance changes in dark adapted *P. meiringensis* 700 μs (•) and 20 ms (○) after the exciting flash.

to the peak position of the main electrochromic transient band in *Chlorella* (Joliot and Delosme 1974), but shifted slightly towards longer wavelengths in comparison to other xanthophyceae (Fork 1969; Fork and Brown 1974). As in these organisms, but in contrast to the transient spectra in green algae (Lavergne et al. 1984), the negative band peaked at 495 nm, and not at 478 nm. A shoulder could clearly be resolved at 540 nm in *P. meiringensis*. A similar shoulder was observed in a mutant of *Chlorella sorokiniana* lacking the CP I-complex in the presence of DCMU and ascribed to an electrochromic band shift of pheophytin due to the accumulation of Q_A^- (Lavergne et al. 1984). However, in *P. meiringensis* this shoulder was observed in untreated wild-type cells. In another xanthophyceae, *Tribonema aequale*, Fork and Brown (1974) observed a clear maximum at 537 nm, the origin of which was not identified. Whereas in *T. aequale* the peak at 537 nm was the predominant absorbance change in that part of the transient spectrum, it was lacking in another xanthophyceae, *Botrydiopsis alpina* (Fork 1969). Thus, the origin of this band remains to be determined in these organisms.

The initial amplitude of the absorbance change at 515 nm was decreased by about 60% upon addition of 20 μM DCMU (data not shown), a sensitivity similar to that of the electrochromic absorbance changes in higher plant leaves (Garab et al. 1983). The transient spectrum between 505 and 545 nm did not change in shape (data not shown). These data supported the notion that the absorbance change around 520 nm is due to an electrochromic shift of pigments in *P. meiringensis*.

In dark-adapted cells of *P. meiringensis* the rereduction of Cyt f^+ occurred with about the same relatively slow time course as the other absorbance transients between 450 and 540 nm. In order to separate the signal due to redox changes of Cyt *f* from the transients between 450 and 540 nm, different preillumination protocols were tested to find a condition where the rereduction of cytochrome and the decay of the 515 nm signal differed kinetically. Preillumination of the cells 600 ms prior to each measurement by four actinic flashes spaced by 100 ms led to a good separation of the signals at 515 nm and 553 nm: it accelerated the rereduction of Cyt f^+, without significantly changing the decay at 515 nm (data not shown). By subtracting the contribution of the Cyt f^+, the transient between 490 and 560 nm could reasonably well fitted with a first derivative of a gaussian band peaking at 506 nm (data not shown). This supported the notion that the transient between 490 and 540 nm originate mainly in electrochromic shift.

The transient spectrum of dark adapted cells between 450 and 540 nm was fitted with first derivatives of gaussian bands (Fig. 3). Three absorption bands could be deconvoluted in the region between 440 and 540 nm. The fit was started between 500 and 530 nm (Fig. 3A). The first calculated band peaked at 506 nm in accordance with the fit obtained after subtracting the contribution of the cytochromes. Probably due to spectral overlap by the main band the second fit was less precise than the first one as was obvious by comparing the scatter of data points in Fig. 3A and 3B. The fit of the last band (Fig. 3C) carried some further uncertainty as contributions from cytochromes and $P700^+$ in this spectral region (Bouges-Bocquet 1977; Hiyama and Ke 1971) could not be excluded. As shown in Fig. 3D the calculated transient spectrum between 450 and 540 nm fitted reasonably well the measured data points.

Based on the fitted values of ν_0, σ and k, the absorbance spectrum of the shifted pigment(s) was reconstituted (Fig. 4). It must, however, be noted that the calculated absorbance spectrum does not necessarily follow the absorbance of the pigment(s), since the fitted amplitudes depend not only on the extinction coefficient but also on the magnitude of the band-shifts. Furthermore, overlaps from other minor electrochromic or non-electrochromic bands could not be ruled out.

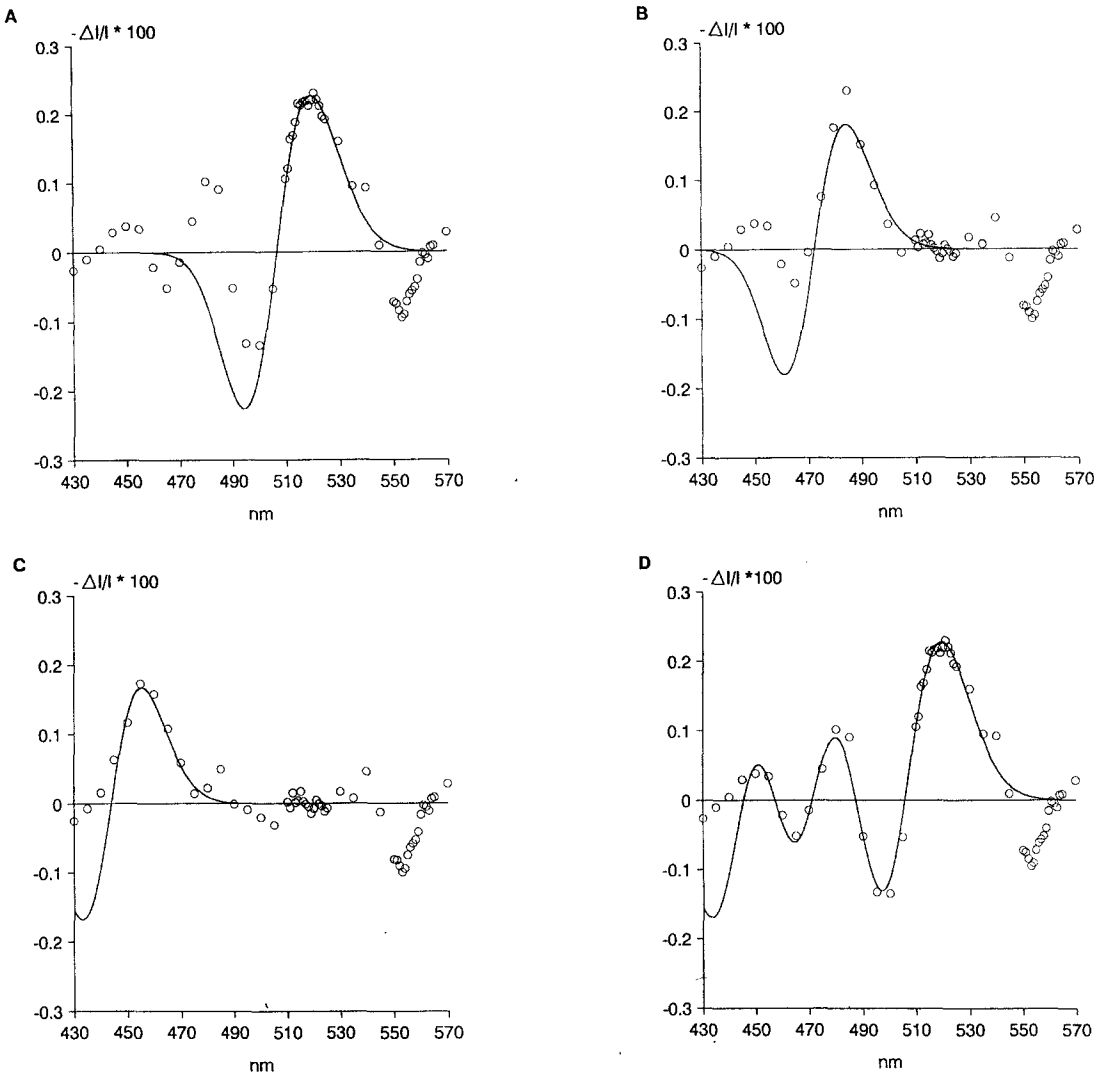

Fig. 3. Mathematical fit of the spectrum of fast absorbance changes of dark adapted *P. meiringensis* 700 μs after the flash. Curves in (a)–(c) show consecutive fittings, (d) the calculated spectrum; symbols represent measured data points (a, d) and values obtained after subtracting the fitted values (b, c), respectively (see Material and methods). The fitting parameters obtained by iteration are as follows: (a) $\nu_0 = 5.921 \cdot 10^{14}$ s^{-1} (= 506 nm), $\sigma = 1.500 \cdot 10^{13}$ s^{-1} and $k = 2.100 \cdot 10^{26}$ s^2; (b) $\nu_0 = 6.349 \cdot 10^{14}$ s^{-1} (= 473 nm), $\sigma = 1.575 \cdot 10^{13}$ s^{-1} and $k = 1.850 \cdot 10^{26}$ s^2 and (c) $\nu_0 = 6.757 \cdot 10^{14}$ s^{-1} (= 444 nm), $\sigma = 1.700 \cdot 10^{13}$ s^{-1} and $k = 2.000 \cdot 10^{26}$ s^2.

The existence of the 506 nm absorbance band could also been demonstrated in whole cells with the aid of fluorescence excitation spectra. As shown by monitoring low temperature fluorescence emission of PS II (688 nm) and PS I (715 nm) the band absorbing at 506 nm transfers energy to both PS II and PS I (Fig. 5).

Discussion

Light-induced transient absorbance spectra have been published for a variety of organisms including the heterokontae, such as phaeophyceae, diatoms, eustigmatophyceae and xanthophyceae (Fork 1969; Fork and Amesz 1967; Fork and Brown 1974). The spectra measured in xanthophyceae and eustigmatophyceae closely resemble that of *P. meiringensis* in the shorter wavelength range (430–500 nm) with regard to the position of maxima (≈ 450 nm, ≈ 482 nm) and minima (≈

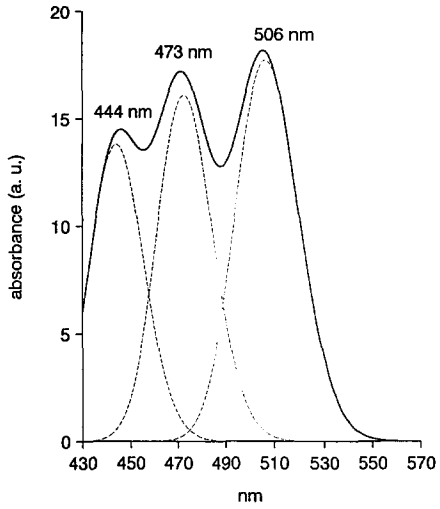

Fig. 4. Absorbance bands resulting from consecutive fits of transients as shown in Fig. 3 (.....) and sum of bands (—) of the pigment molecule(s) undergoing electrochromic shift in *P. meiringensis*.

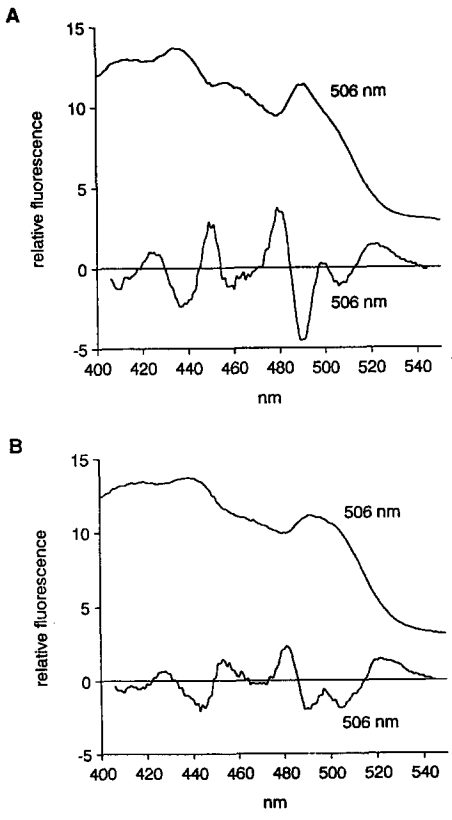

Fig. 5. Fluorescence excitation spectra at 77 K and second derivatives of the spectra recorded at the emission wavelength 688 nm (A) and 715 nm (B).

465 nm, ≈ 496 nm). Especially the spectrum of the xanthophyceae *Botrydiopsis alpina* shows large similarities to that of *P. meiringensis* also in amplitude. The main differences are found around 520 nm. In *P. meiringensis* the transient spectrum is dominated by the 520 nm band, whereas in *Tribonema aequale* this band is present only as a shoulder and the 537 nm transient band is the most intense of all bands between 450 and 550 nm. This peak is also apparent in *Pleurochloris magna*, an alga belonging to the eustigmatophyceae because it lacks Chl-*c*. Nevertheless, as the reported spectra were measured with long (0.3–3 s) flashes, a comparison to our data obtained with single turnover flashes is not straightforward.

As the transients of flash-induced absorbance changes in the xanthophyceen alga *P. meiringensis* were observed 700 μs after excitation, they cannot be assigned to redox alterations of P700 and the Cyt b_6/f complex for spectral and kinetical reasons (Hiyama and Ke 1971; Wasserman 1980). Relaxation of carotenoid triplets after a flash should be faster than our time resolution and can therefore also be neglected (Mathis and Vermeglio 1975). Furthermore, fast scattering transients are unlikely to contribute with large amplitudes, because in green algae they have been shown not to distort significantly the fast transient spectra of absorbance (Garab et al. 1978).

In green algae and higher plants, fast absorption changes around 515 nm originate mainly from electrochromic response of pigment molecules embedded in the thylakoid membrane (Junge and Witt 1968). The transients in *P. meiringensis* exhibit comparable spectral and kinetical behaviour as published for these organisms (Bouges-Bocquet 1977; Garab et al. 1989; Joliot and Delosme 1974; Joliot and Joliot 1992; Lavergne et al. 1984) favouring the idea of an electrochromic bandshift of pigments in the thylakoid membrane of *P. meiringensis*.

The fact that DCMU decreased the initial amplitude of ΔA520 by about 60% further indicates that the observed changes are of electrochromic nature. PS II and PS I contribute equally to the generation of the transmembrane field (Junge and Jackson 1982). Thus, the suppression of the signal by DCMU is in reasonable accordance with the measured PS II/PS I ratio of 2.88 in *P. meiringensis* (Büchel et al. 1988). In higher plant chloroplasts most of the slowly recovering PS II reaction centres remain undetected at a flash frequency of 1 s^{-1} (Chylla et al. 1987). This might explain the underestimation of PS II/PS I ratio by electrochromic absorbance changes compared to other methods.

Electrochromic absorbance changes can be unambiguously identified in isolated thylakoids on the basis of their sensitivity to ionophores which accelerate the decay of the transmembrane potential difference (Junge and Witt 1968). The electrochromic nature of the changes at 700 μs in *P. meiringensis* could not be tested with ionophores, because they do not penetrate intact cells. Attempts to isolate intact chloroplasts have not been successful. Thus, the origin of absorbance changes in Chl-*c*-containing algae was analysed on the basis of mathematical deconvolution of the transient spectrum. A well established feature of electrochromism is that the absorbance bands of the pigments which detect the field undergo a homogeneous shift (Junge 1977). Thus, the transient bands can be approximated with first derivatives of gaussians. With mathematical fitting we have shown that the transients between 450 and 540 nm in *P. meiringensis* can indeed be fitted with suitable precision by a linear combination of derivative bands. Our analysis shows that the changes can be attributed to a homogeneous shift of three absorbance bands with peak positions at 444 nm, 473 nm and 506 nm with halfbandwidths of 27.5 nm, 28.5 nm and 30.5 nm, respectively. The peak positions suggest that Chl-*c* and Chl-*a* do not contribute significantly to the major absorbance changes. Taking into account the band structure of carotenoids of *P. meiringensis* (Stransky and Hager 1970) it appears more likely that the three bands can be assigned to the same carotenoid molecule. The tenfold smaller amplitude of the transient spectra at 520 nm compared to that measured in green algae (Joliot and Delosme 1974; Bouges-Bocquet 1977) might be due to the fact that the cells were used at a similar chlorophyll content but not at a comparable carotenoid concentration since the ratios of different carotenoids to chlorophyll in thylakoids of *P. meiringensis* are 2 to 6.25 fold lower (Büchel et al. 1988) in comparison to the sum of Chl-*b* and lutein in that of higher plants (Bassi et al. 1993). Nevertheless, the smaller signal might also be due to differences in the extinction coefficients of the pigments or in the extent of the homogeneous shift, respectively.

As demonstrated by the fluorescence excitation spectra, a carotenoid with an absorption maximum at 506 nm is present in *P. meiringensis* and transfers energy to both PS I and PS II. From the spectra of isolated pigment-protein complexes (Büchel and Wilhelm 1993) it is obvious that this pigment is bound both to LHC II and LHC I, but not to the PS I core complex. β-carotene, which is the carotenoid absorbing at longest wavelength in organic solvent is absent from the light-harvesting complexes of *P. meiringensis* and thus cannot be responsible for the absorbance changes. Heteroxanthin and diatoxanthin are present both in LHC II and LHC I (Büchel and Wilhelm 1993) and in organic solvents they both have an absorption at longer wavelength than diadinoxanthin and vaucheriaxanthin-ester. Since, however, the in vivo absorbance of these carotenoids is not known the exact identification of the pigment molecule(s) which exhibit the homogeneous shift upon flash excitation in *P. meiringensis* is presently not possible.

Acknowledgements

The authors kindly acknowledge a grant for C. Büchel from the Deutsche Forschungsgemeinschaft (436 UNG-113/96/1). This work was also supported by a grant, OTKA III/2999 from the Hungarian Research Fund. We are grateful to Dr Wilhelm for fruitful discussions.

References

Barabás K, Zimányi L and Garab G (1985) Kinetics of the flash-induced electrochromic absorbance change in the presence of background illumination. Turnover rate of electron transport. II. Higher plant leaves. J Bioenerg Biomembr 17: 365–373

Bassi R, Pineau B, Dainese P and Marquardt J (1993) Carotenoid-binding proteins of Photosystem II. Eur J Biochem 212: 297–303

Böger P (1969) Photophosphorylierung mit Chloroplasten aus Bumilleriopis filiformis Vischer. Zeitschrift für Pflanzenphys 61: 85–97

Bouges-Bocquet B (1977) Cytochrome *f* and plastocyanin kinetics in *Chlorella pyranoidosa*. I. Oxidation kinetics after a flash. Biochim Biophys Acta 462: 362–370

Büchel C and Garab G (1994) Evidence for the operation of a cyanide-sensitive oxidase in chlororespiration in the thylakoids of the chlorophyll *c*-containing alga *Pleurochloris meiringensis* (Xanthophyceae). Planta (in press)

Büchel C, Wilhelm C and Lenartz-Weiler I (1988) The molecular analysis of the light adaptation reactions in the yellow-green alga *Pleurochloris meiringensis* (Xanthophyceae). Botanica Acta 101: 306–310

Büchel C and Wilhelm C (1993) Isolation and characterization of a Photosystem I-associated antenna (LHC I) and a Photosystem I-core complex from the chlorophyll *c*-containing alga *Pleurochloris meiringensis*. J Photochem Photobiol 20: 87–93

Chow WS, Melis A and Anderson JM (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proc Natl Acad Sci USA 87: 7502–7506

Chylla RA, Garab G and Whitmarsh J (1987) Evidence for the slow turnover in a fraction of Photosystem II complexes in thylakoid membranes. Biochim Biophys Acta 894: 562–571

Cramer WA, Black MT, Widger WR and Girvin ME (1987) Structure and function of photosynthetic cytochrome *b*- and b_6f-complexes. In: Barber J (ed) The Light Reactions, pp 447-493. Elsevier Science Publishers, Amsterdam

Duysens LNM (1954) Reversible changes in the absorption spectrum of Chlorella upon irradiation. Science 120: 353–354

Fork DC (1969) Evidence for the participation of carotenoids in the photosynthesis of algae and higher plants. In: Metzner H (ed) Progress in Photosynthesis Research, pp 800–810. International Union of Biological Science, Tübingen

Fork DC and Amesz J (1967) Light-induced shifts in the absorption spectrum of carotenoids in red and brown algae. Photochem Photobiol 6: 913–918

Fork DC and Brown JS (1974) A comparison of light-induced shifts in carotenoid absorption in representatives of different algae groups. Carnegie Inst Wash Yearbook: 776–779

Garab G, Lajkó F, Mustárdy L and Márton L (1989) Respiratory control over photosynthetic electron transport in chloroplasts of higher plant cells: Evidence for chlororespiration. Planta 179: 349–358

Garab G, Paillotin G and Joliot P (1978) Flash-induced scattering transients in the 10 μs–5 s time range between 450 and 540 nm with Chlorella cells. Biochim Biophys Acta 545: 445–453

Garab G, Sanchez Bargos AA, Zimányi L and Faludi-Dániel A (1983) Effect of CO_2 on the organization of thylakoids in leaves of higher plants. FEBS Lett 154: 323–327

Girault G and Galminche JM (1976) Nucleotides effect on the decay kinetics of the 520 nm absorbance change in tightly coupled chloroplasts. Biophys Res Comm 68: 724–729

Hiyama T and Ke B (1971) A further study of P430: A possible primary electron acceptor of Photosystem I. A Biochem Biophys 147: 99–108

Hope AB, Huilgol RR, Pamizza M, Thompson M and Matthews DB (1992) The flash-induced turnover of cytochrome *b*-563, cytochrome *f* and plastocyanin in chloroplasts. Models and estimation of kinetic parameters. Biochim Biophys Acta 110: 15–26

Houchins JP and Hind G (1983) Flash-spectroscopic characterization of photosynthetic electron transport in isolated heterocysts. Arch Biochem Biophys 224: 272–282

Jeffrey SW and Humphrey GF (1975) New spectrometric equations for determining chlorophyll *a*, *b*, *c*1 and *c*2 in higher plants, algae and natural phytoplankton. Biochem Biophysiol Pflanzen 167: 191–194

Joliot P and Delosme R (1974) Flash-induced 519 nm absorption change in green algae. Biochim Biophys Acta 357: 267–284

Joliot P and Joliot A (1989) Characterization of linear and quadratic electrochromic probes in *Chlorella sorokiniana* and *Chlamydomonas reinhardtii*. Biochim Biophys Acta 975: 355–360

Joliot P and Joliot A (1992) Electron transfer between Photosystem II and the cytochrome *b/f* complex: Mechanistic and structural implications. Biochim Biophys Acta 1102: 53–61

Junge W and Jackson JB (1982) The development of electrochemical gradients across photosynthetic membranes. In: Govindjee (ed) Photosynthesis, Energy Conversion by Plants and Bacteria, Vol 1, pp 590–639. Academic Press, New York

Junge W and Witt HT (1968) On the ion transport system of photosynthesis – investigations on a molecular level. Z Naturforsch 23: 244–254

Junge W (1977) Membrane potentials in photosynthesis. Ann. Rev. Plant Physiol 28: 503–536

Lavergne J, Delosme R, Larsen U and Bennoun P (1984) Mutants of *Chlorella sorokiniana*: A new material for photosynthesis studies. II. Improved spectroscopic analysis of electron transfer in mutant strains. Photobiochem Photobiophys 8: 207–219

Mathis P and Vermeglio A (1975) Chlorophyll radical cation in Photosystem II of chloroplasts. Millisecond decay at low temperature. Biochim Biophys Acta 396: 371–381

Mitchell P (1974) A chemiosmotic molecular mechanism for proton translocating adenosine triphosphatases. FEBS Lett 43: 189–194

Morita S, Itoh S and Nishimura M (1983) Flash induced photophosphorylation in chloroplasts with activated ATPases. Biochim Biophys Acta 724: 411–415

Paillotin G and Breton J (1977) Orientation of chlorophylls within chloroplasts as shown by optical and electrochromic properties of the photosynthetic membrane. Biophys J 18: 63–79

Sandmann G and Böger P (1980) Physiological factors determining formation of plastocyanin and plastidic cytochrome *c*553 in Scenedesmus. Planta 147: 330–334

Schmidt S, Reich R and Witt HT (1971) Electrochromism of chlorophylls and carotenoids in multilayers and in chloroplasts. Naturwissenschaften 58: 414–415

Schreiber U and Rienits KG (1982) Complementarity of ATP-induced and light-induced absorbance changes around 515 nm. Biochim Biophys Acta 682: 115–123

Sewe KU and Reich R (1977) The effect of molecular polarization on the electrochromism of carotenoids. II. Lutein-chlorophyll complexes: The origin of the field-indicating absorption change at 520 nm in the membrane of photosynthesis. Z Naturforsch 32: 161–171

Stransky H and Hager A (1970) The carotenoid pattern and the occurrence of the light induced xanthophyll cycle in various classes of algae. Arch Microbiol 71: 164–190

Wasserman AR (1980) Chloroplast cytochromes *f*, *b*-559 and b_6. Meth Enzymol 69: 181–202

Wild A, Höpfner M, Rühle W and Richter M (1986) Changes in the stoichiometry of Photosystem II components as an adaptive response to high-light and low-light conditions during growth. Z Naturforsch 41: 597–603

Witt HT (1955) Kurzzeitige Absorptionsänderungen beim Primärprozeß der Photosynthese. Naturwissenschaften 58: 414–415

Witt HT (1979) Energy conversion in the functional membrane of photosynthesis. Analysis by light pulse and electric pulse methods. The central role of the electric field. Biochim Biophys Acta 505: 355–427